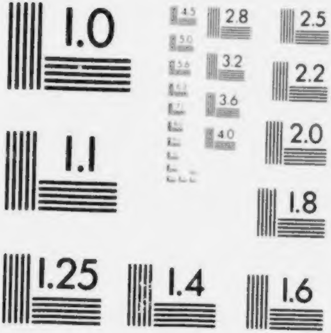
MICROCOPY RESOLUTION TEST CHART

(ANSI and ISO TEST CHART No. 2)



SUPPLEMENTARY OBSERVATIONS ON THE DEVELOPMENT OF THE CANADIAN OYSTER

J. STAFFORD, M.A., Ph.D.

NEW YORK
1912

[Reprinted, without change of paging, from the American Naturalist, 1912.]

[Reprinted from THE AMERICAN NATURALIST, Vol. XLVI., Jan., 1912.]

SUPPLEMENTARY OBSERVATIONS ON THE DEVELOPMENT OF THE CANADIAN OYSTER

J. STAFFORD, M.A., PH.D.

BIOLOGICAL STATION, DEPARTURE BAY, B. C.

IN the AMERICAN NATURALIST of January, 1905, January, 1909, June, 1910, I have given some account of observations (in 1904) on the development of the oyster at Malpeque, Richmond Bay, Prince Edward Island, Canada.

Opportunity to verify, continue, and extend these observations was again afforded in 1909, when I studied the oyster in the most important centers along the east coast of New Brunswick.

In the present summer, 1911, being occupied at the Pacific Biological Station of Canada, in Departure Bay, near Nanaimo, Vancouver Island, I have the privilege of observing some of the Prince Edward Island oysters transplanted to this vicinity in 1905, as well as adding to my acquaintance the little British Columbia oyster, so different in size, appearance, habits and reproduction.

In the intermediate years, not being located in oyster regions, I devoted a good deal of time to other bivalve-larvæ, largely with a view to making my studies of the oyster more secure, the main results of which have been given in a paper "On the Recognition of Bivalve Larvæ in Plankton Collections," unreasonably delayed in publication at Ottawa.

In all this work I have kept sample preservations with dates and localities, which have often proved of great service in judging of questions that subsequently arose.

My first work began where that of Brooks left off, and showed for the first time that later stages of the oyster-larva undoubtedly exist, and when, where and how they

may be procured, as well as the length of the period of their free-swimming life. The larvæ obtained by Brooks, Rice, Ryder, Winslow, and others were obtained by culture from fertilized eggs, and were at most six days old, and in the young straight-hinge stage. In Europe larvæ of a similar age, size and structure had been taken from the infra-branchial cavity of the parent oyster by Davaine, Lacaze-Duthiers, Costé, De la Blanchère, Gwyn Jeffries, Saunders, Salensky, Möbius, Horst and Huxley, but the older, later or larger stages were quite unknown. This left room for some speculation as to the exact time, place and manner in which the succeeding stages should be found, as well as occasioned the prevalent mistake that the free larva settles down at this period to become a fixed spat. Brooks wrote, "All my attempts to get later stages than these failed . . . and I am therefore unable to describe the manner in which the swimming embryo becomes converted into the adult, but I hope that this gap will be filled, either by future observations of my own or by those of some other embryologist." In a similar way Jackson, at a later period, speaks of "a blank in the knowledge of the development of the oyster." This "gap" or "blank" is now completely filled. My studies prove that the larva continues to live as a larva in the sea-water about oyster-beds for two or three weeks longer, where it swims about, feeds, grows and changes in structure, and that it first settles down to become a sedentary spat, fixed to shells or other objects, at an age of three to four weeks from fertilization—the length of time depending to some extent on temperature, food, individuality or such causes. This information has been gained through the method of procuring oyster-larvæ from the waters of oyster-areas by means of a plankton-net, and connecting them in series with younger stages obtained by fertilization and culture and with older stages obtained by catching spat on glass, shells, etc., so as to make out the complete life-history.

The discovery that the hitherto unknown stages of the

oyster-larva can be conveniently obtained by a plankton-net carries with it the possibility of a practical application of inestimable value in the culture of oysters. From the time of the early Roman Empire it has been known that oyster-spat can sometimes be obtained on ropes, anchors, piles of wharves, stones, shells or other natural or artificial objects in the sea, and some sort of method of culture has long been in use in many countries. At times men have risen to exalted conceptions of the possibility of finding a practicable, safe and sure method of catching, retaining, and rearing the young spat. I quote Winslow to the effect that "Thousands of dollars would be annually saved by the Connecticut oystermen if they could determine, with even approximate accuracy, the date when the attachment of the young oyster would occur. Hundreds of thousands would be saved if they had any reliable method of determining the probabilities of the season." This is now possible.

It is well known that oyster or other shells dried and whitened in the sun form the very best oyster-collectors or cultch. To put these back into the water haphazard has often resulted solely in the loss of all the labor of preparation. In even a few days they may become covered with a slimy coating which reduces or largely destroys their efficiency. The point is to be able to determine with accuracy, for each season and for every locality, when oyster-larvæ are present in the water full-grown and ready to settle as spat, so as not to run the risk of losing adequate value for the laboriously prepared cultch. A man instructed and qualified in the method of taking plankton and in identifying oyster-larvæ can tell almost to a day when is the proper time to put out cultch so as to obtain an abundant and copious set of spat. It is not enough to know about the time, or to know the time for certain previous years, or to know the average time.

Three methods are open to the expert: (1) Examination of the genital organs of adult oysters to determine

when the eggs are ripe, (2) examination of the sea-water to learn if oyster-larvæ are present and in what stage, (3) examination of natural or improvised objects in the water to discover if young spat are already formed. The first is not immediately determinative because of the long period of development separating spawning and spatting. The last is not very practicable because of the difficulty of finding and recognizing the youngest spat before the period is gone by for putting out cultch. The second is the only practicable and conclusive method and its efficiency is proportionate to the number, care and accuracy of the observations. Its success will increase with experience.

This method makes use of the colossal number of larvæ lavishly provided by nature to offset the exigencies and accidents of life and insure a reasonable chance of keeping up the stock. I believe that all the larvæ an army of men could raise up and turn into the sea would not materially alter the number of successful individuals in the set of spat. But on the other hand a few culturists could enormously increase the chances for a successful catch by spreading an abundance of suitably prepared cultch at the proper time and place.

In the paper of 1909 I have described the method of obtaining plankton, the appearances and measurements of the oyster-larvæ to be recognized, the time of the year to begin making observations. In the paper on "Bivalve Larvæ" I distinguish in sizes, shapes, colors, the commonly occurring associates of the oyster-larvæ which might be taken for the latter. In the present paper, after long reflection, I suggest a practical application of the knowledge acquired.

I should not omit to mention that the paper of 1910 connects the larva, through the youngest microscopic spat, with the macroscopic spat of fishermen and finally with the adult. Similarly in 1909 I performed extensive artificial-fertilization experiments, while at Shediac, Caraquette and Malpeque, in order to connect the small-

est plankton stages of oyster-larvæ with culture-stages and through these back to the egg. Larvæ by the million were reared in beakers of sea-water at a temperature little above 20° C. and with a specific gravity (salinity) varying somewhat under 1020. I also carried Caraquette oysters to Malpeque and raised up larvæ from eggs cross-fertilized between two such obviously different varieties as the small, narrow, curved, thick, hard and heavy Caraquette oyster and the fine, large, broad, straight, clean, smooth specimens from the Curtain Island beds.

In 1896 and again in 1905 the Canadian Government had Atlantic oysters transshiped to the Pacific and put out at selected places. In the latter year some of the places were chosen by Captain Kemp, expert in oyster culture.

Being occupied this summer at our Pacific Biological Station, I have taken advantage (although not requested to do so) of my proximity to three of these places to search for the transplanted Prince Edward Island oysters, and to examine plankton taken in the vicinity. At the first place, Hammond Bay, being a small bay and close to hand, I could easily over-run all the beach at low water, and soon discovered the dead shells that had been deposited too far above low-water mark. At Nanoose Bay, some twelve miles away, perhaps five miles long and a mile and a half wide, with extensive flats at low tides, this was not so easily done. Having spent three summers with Captain Kemp, I thought now to test my judgment of where he would select to deposit the oysters. As the tide was unfavorable at my first visit I used the dredge, and was afterwards surprised to learn that I had actually calculated to within a few rods of the place. At the second visit I went to look at other parts of the bay, but on the third returned and, with a favorable tide, could wade and pick up some of the oysters. This was at 3 P. M., July 17, and I took 16 fine living specimens of the Malpeque oyster for examination—two or three of them with pieces of Prince Edward Island red-sandstone still

attached to them. They varied from two and three fourths to five inches in length, some of them showing considerable growth. This proves that Atlantic oysters can be transplanted to the Pacific and remain healthy and grow. Upon reaching home I proceeded to examine some of the oysters and it turned out that only one had already spawned while the other fifteen were ripe and generally somewhat distended with eggs or sperm. This proves that the transplanted oysters can come to maturity and ripen the reproductive elements.

At 7.10 P. M. of the same day I put together eggs and sperm in a tumbler of sea-water and at 7 A. M. next morning there was an abundance of segmentation stages and free-swimming larvæ. This proves that the oysters can spawn and that the eggs can develop into young. I make these statements because of a prevailing opinion that the transplanted oysters have all died, and the few people who think there are still some living are dogmatic in their assertion that they do not breed.

Plankton taken at intervals at Hammond and Nanoose Bays had not yielded any oyster larvæ, which became explainable upon finding the condition of the reproductive organs. A further observation on this was afforded on the 26th of July, when I examined a second lot (obtained at a very low tide the day before) from Nanoose Bay. The forty-seventh oyster examined was the first to yield good ripe eggs—all previous ones were spawned with the exception of four or five which were ripe males. The interval between these two visits had been the hottest of the summer and the oysters had nearly all spawned in this period—slightly later than is usual on the Atlantic. On the 27th I made a trip to Oyster Harbor (Ladysmith), about fifteen miles from here, where I had better luck in getting track of the few transplanted oysters. In a similar way I examined several individuals and took plankton which for the first time contained larvæ of the Atlantic oyster—recognizable by their shape and measurements but not presenting such a deep pink or brown

coloration as in their native home. For comparison with my former papers I will give the measurements of a single specimen with the characteristic postero-dorsal high umbos, the large convex left valve, and the smaller and flatter right valve, velum, foot, pigment spot and the rest. Ocular V, objective 4, 42 long by 37 high ($=.289 \times .255$ mm.). This proves that larvæ grow up. There is only one other bit of evidence possible and that is to find spat. This I have not done as yet. It is too early for this year's spat and I have not seen any undoubted specimens of a former year's spat. One can judge that the comparatively few descendants of two and a half barrels deposited at Hammond Bay, five barrels at Nanoose Bay, and one barrel at Oyster Harbor, when dispersed over the broad areas at their command, would not prove very conspicuous objects, which is again complicated by the presence of millions of British Columbian oysters of varying sizes, shapes, and complexions.

I regard my findings as conclusive and would urge the transplanting of Atlantic oysters (*Ostrea virginica* Gmel.) to the Pacific in greater quantities. The Atlantic clam (*Mya arenaria* L.) has propagated enormously here notwithstanding the fact that it has more competitors in its particular habit than in its original home.

Ostrea lurida Carp.—Even before making any headway in the foregoing researches, I had begun to gather information on the occurrence, size, shape, color, structure, breeding, etc., of the British Columbia oyster.

This species is not common in Departure Bay, or in Hammond Bay, but a few specimens may be found under stones exposed at about one hour from low water in front of the C. P. R. cable house in the former, and just inside the far point of the latter, and are usually so broadly and solidly attached (with the left valve against the under side of the stone and hence uppermost) that it is scarcely possible to separate them without destroying

the attached surface. But on the extensive flats at the upper ends of Nanoose Bay and of Oyster Harbor they occur free on the surface by thousands and more or less covered with barnacles.

Good specimens reach two inches in length by an inch and a half in breadth, with a straight dorsal margin and a semicircular ventral curvature. The right, upper or smaller valve is nearly flat or but little convex and fits into the margins of the larger, convex, lower or left valve, the greater part of the lower and posterior margin being scalloped, while the left valve has corresponding ridges and points. The color is usually dark (those under stones lighter) with the older parts weathered grayish and the umbonal region of the left valve is often attached to a small stone or another oyster or bears a scar. Internally the shell is extensively pigmented, dark, with smaller bands or blotches of lighter pearl, while the muscle scar is rather lighter and banded. The mantle is broadly margined with dark, which may also creep up on to the abdomen.

The most interesting feature in connection with the Pacific oyster of Canada is its divergence in some respects from the mode of breeding of our Atlantic species. In the British Columbia form there is no primary separation of individuals into males and females—the sexes are united in each individual. In other words each individual is bisexual, monœcious or hermaphrodite. In this respect it is identical with the English or common European species (*Ostrea edulis* L.).

My first observations were made on July 12, on specimens procured under stones near the Biological Station. Nearly all appeared to be males, and, as they were of small size, I took it that, as commonly occurs, the males had ripened earliest. But one was of medium size and contained eggs that at once attracted my attention on account of their large size, opacity and rare exhibition of nucleus. Measured exactly as all my former measurements, these gave: Oc. V, obj. 2 = 6.5; Oc. V,

obj. 4 = 15; Oc. V, obj. 7 = 72. Another individual, obtained since, with an abundance of eggs oozing from the oviduct, pure and ripe, gave the almost unvarying measurement of the egg as: Oc. V, obj. 7 = 75. This when calculated is $75 \times 1.45\mu = 108.75\mu$ = slightly over .1 mm. = slightly over $\frac{1}{250}$ inch = fully twice the diameter of the egg of the Atlantic oyster, and perhaps identical in size with the egg of the English oyster.

In making measurements it is important to use only ripe eggs, as in this case, and to select those that are spherical or nearly so and not flattened by the weigth of the coverslip, as well as to extend the measurements to many individuals in order to exclude all possibility of a slip. The nucleus is between one half and two thirds the diameter of the egg.

Upon turning particularly to spermatozoa I found them in every individual—even between the eggs of those containing eggs in the gonad. The younger individuals had no ova, but all sperms. Some of the older ones had a few big, soft, opaque, irregular, elliptical, oval or nearly spherical eggs, scattered among irregular masses of less than half their size, which are balls of spermatids on the way to development into spermetozoa. One of these measured $46\mu \times 40\mu$, and each one is kept in a dancing or rolling movement, somewhat like that of many infusoria, by the flapping of the tails of the ripening sperms on the surface. Between these masses are millions of mature, free, dancing spermatozoa, of which the tails are rarely visible until one searches for them with a high power. I have not yet made extensive measurements of the sperm on account of the difficulty of measuring such exceedingly small objects with certainty, but I believe the sperm of the British Columbia oyster is smaller than that of the Prince Edward Island oyster, which may have some relation to the particular mode of fertilization, such as being introduced by the respiratory current. In some parts of the gonad ova may be plentiful, while at other parts there are only sperm-balls.

Later, in the warmer weather, the sperm may be pretty well run off and the reproductive organ contain mostly eggs. In this way the younger oysters, and the older oysters at the beginning of the season, may be physiologically males, while older oysters at the height of the breeding season may be physiologically females.

Oysters from Hammond Bay showed the same phenomena.

Upon finding an abundance of larger oysters on the surface at Nanoose Bay, I brought home a pail-full of picked specimens to serve as a convenient stock for observation and experiment. On July 16 I found a specimen with perhaps half a teaspoonful of eggs in various stages of segmentation, lying free in the lower valve—a mass of white granules. The ripe eggs ooze into the infra-branchial cavity and lie on and between the gills, *i. e.*, between the two folds of the mantle, where they are retained apparently without any retaining, sticky matrix. I suppose that it is here they first meet with ripe sperms from other individuals, for I do not believe that at this time the sperms of the same individual are physiologically capable. The whole oyster appears exhausted, the gills rent, the flesh collapsed, soft and parts of it almost rotten. On July 24 I opened one hundred of the stock supply and found six with eggs, embryos or conchiferous young, in the infra-branchial cavity. All the others were in process of spermogenesis and oogenesis.

An experiment that has often seemed possible to me is to do the same with the European oyster, by way of artificial fertilization, as Brooks did with the American oyster. Now that I had an oyster essentially the same as the European I tried it, and with seeming success, but of course it is difficult to be sure that sperm from another had not already had access to the eggs. Unripe eggs are no good; eggs already freed from the gonad may have come in contact with sperm. This restricts one to finding a specimen just before but just on the point of

extruding its eggs. I also tried Atlantic oyster eggs with Pacific oyster sperms, as well as Atlantic oyster sperms with Pacific oyster eggs, but without success, as one might suppose. I put eggs, embryos and larvæ of both species together under the same coverslip for comparison—those of the small British Columbia oyster looking like giants beside those of the large Prince Edward Island oyster. This is a curious phenomenon which I have several times observed on other species, *e. g.*, the very large eggs of Astarte compared with the small eggs of large species like Mactra.

For the study of segmentation, etc., the Atlantic species is of advantage on account of smaller size and greater transparency. The order of segmentation appears to be the same in both—both subject to variations such that it would require a great number of painstaking observations to decide exactly what is the normal mode in good healthy eggs. I have, on both sides of this continent, spent considerable time in trying to determine the order of segmentation, the cell-lineage, the planes of cleavage, the succession of nuclei, the effect of gravitation, the constant and continuous orientation of successive stages, the origin of the shell-gland and the mode of formation of the shell, etc., but can not discuss such subjects here. I may briefly state, however, that I believe Brooks failed to observe the shell-gland, in his original work, and at one particular stage mistook the relation of the shell-valves to the blastopore which made it necessary to reverse his orientation of the embryo—hence his use of the terms dorsal and ventral are misleading. The polar bodies are dorsal at first—later, if they persist, they may become displaced anteriorly. The blastopore is ventral, the velum anterior, the shell-gland dorsal, the mouth ventral. There is no foot, nor rudiment of it, in pre-conchiferous stages.

I have found conchiferous young of the British Columbia oyster retained within the parent's shell until their own minute shells were .138 mm. in length. I believe

they remain longer, for, according to Möbius, the young of the European oyster leaves the parent at a size of .15 to .18 mm. (Horst gives .16 mm.; Huxley $1/150$ inch). I have taken larvæ of *O. lurida* in plankton (identified by comparison with those from a parent, and also by the structure, shape and size) of a length of .165 mm. as well as different larger sizes. They still had a straight-hinge line of half the length of the shell—unlike the *O. virginica* which at this size is already passing into the umbo-stage and with a much shorter hinge-line. The larvæ of *O. lurida* are not pink or brown but have five or six dark blotches in the region of the liver and in the velum, in contrast to the general light shade of the rest of the animal.